PED-149-217 (DAPHYTBONE)

Enhancement of bone defect repair by mussel-inspired TiO_2-based nanostructures functionalized with bioactive phytomolecules

Phase 1 summary-

Phase objectives:

1. Preliminary studies to evaluate the *in vitro* effects of EpF's on cell behavior
2. Synthesis and morphological characterization of nanostructured Ti surfaces, deposition of PDA and NDA on TiO_2 – coated Ti substrates
3. Functionalization and characterization of Ti / TiO_2 substrates covered with PDA / NDA
4. In vitro biological performance assessment of PDA / NDA coated Ti / TiO2 substrates

The current research is becoming more and more focused on nanoscale changes of biomaterials. Among the different methods used, anodizing is a promising strategy to control nanostructure of the oxide layer on the surface of titanium-based materials and to enhance the efficiency of their applications. Another promising strategy for improving the osteointegration of dental and orthopedic implants is deposition of nanofibres by electrospinning, thus mimicking the organization of the extracellular matrix. In the present study, two different approaches to modify the surface of titanium (Ti) substrate have been investigated. These include *(i)* the type of nanometric topography (nanotubes and nanofibres) and *(ii)* the presence of bioactive phytomolecules from *Herba Epimedii* on nanostructured surfaces. In a first stage, 3 molecules derived from the *Epimedium herb* have been studied as functionalizing agents, namely, icariside, icaritine and icariin. Our research has shown that all of these phytomolecules have the ability to promote osteoblastic differentiation and extracellular matrix mineralization. Considering the approximately identical pattern of expression of alkaline phosphatase activity and the similar extracellular matrix mineralization level exhibited by the pre-osteoblasts MC3T3-E1 treated with the aforementioned phytomolecules, icariin was selected as an agent to functionalize the nanostructured surfaces developed in the project. This choice was motivated by its biological action and lower sale price compared to icaritine and icariside.

The optimal conditions for obtaining the TiO_2 nanotubes by anodizing and the nanofibres by electrospinning were established in the experimental approach. The loading of icariin into nanostructures was accomplished by physical adsorption. Samples were characterized by SEM, Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), and Contact Angle Measurements (CA). Their electrochemical characterization has also been carried out. The release of icariin was measured using a UV-VIS spectrophotometer. The pre-osteoblast behavior in terms of viability, morphology, proliferation and cell differentiation, as well as RAW 264.7 macrophage response have been investigated in detail. The results of SEM, FTIR, AFM and CA analyses have shown that nanostructured surfaces loaded with icariin have been successfully obtained. The *in vitro* release profile of icariine from TiO_2 nanotubes and nanofibres revealed sustained release compared to uncoated Ti. Electrochemical characterization studies have demonstrated that both nanostructuring approaches of Ti-based surfaces lead to a visible improvement in corrosion parameters, being obtained high values for corrosion rates (tens nm/year). From the point of view of stability and corrosion resistance displayed by all tested variants, the nanotubes coated and functionalized with polydopamine, nitrodopamine and icariin exhibited the best behavior. Furthermore, the obtained results highlighted the potential of tested nanostructures to positively modulate the bioactivity of osteoblasts. Of the two surface changes, anodization had a greater impact on osteoblast function than electrospinning.

In addition, it has been shown that the coatings developed within the project represent an effective approach to modulate macrophage behavior in order to obtain a response that sustains a healing process rather than the occurrence of chronic inflammation. Together, these findings indicate the potential applicability of the nanostructured surfaces functionalized with icariin in the bone regenerative medicine.